



Antioxidant Trace Metals among Roadside Petrol Dispensers in Gombe State, Nigeria

S. Adamu^{1*}, O. M. Akinosun², F. M. Abbiyesuku², M. A. O. Kuti²,
Jibril M. El-Bashir³ and J. D. Abubakar⁴

¹Department of Chemical Pathology, Federal Teaching Hospital, Gombe, Nigeria.

²Department of Chemical Pathology, University College Hospital, Ibadan, Nigeria.

³Department of Chemical Pathology, Ahmadu Bello University, Zaria, Nigeria.

⁴Department of Community Medicine, College of Medical Sciences, Gombe State University, Gombe, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SA, OMA, FMA and MAOK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JMEB, JDA and SA managed the literature searches. Authors SA and OMA performed laboratory analyses of the samples. The statistical analysis was done by authors SA, JDA and MAOK. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/24251

Editor(s):

(1) Rui Yu, Environmental Sciences & Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, USA.

Reviewers:

(1) Jorge Isaac Castro Bedriñana, National University of the Center of Peru, Peru.

(2) Pawel Pohl, Wroclaw University of Technology, Poland.

(3) Valdecir F. Ximenes, University of the State of Sao Paulo, Brazil.

Complete Peer review History: <http://sciencedomain.org/review-history/13507>

Original Research Article

Received 10th January 2016

Accepted 9th February 2016

Published 2nd March 2016

ABSTRACT

Background: The number of people found to be exposed to gasoline is of public health concern. In developed countries, unlike developing countries, measures are taken to dispense gasoline quickly and safely to reduce exposure. In Nigeria, roadside dispensers are a common sight, often with no any form of protection. This leads to high levels of exposure with the consequences including oxidative injuries. As cofactors in antioxidant reactions, antioxidant trace metals are consumed in the process of converting the free radicals generated by petrol.

Methods: Antioxidant trace metals (zinc, manganese, copper, and selenium) were compared between 90 road side dispensers of gasoline and 90 matched controls. Plasma zinc, selenium, manganese and copper were analyzed using Atomic Absorption Spectrophotometer (AAS).

*Corresponding author: E-mail: sanigombe@yahoo.com;

Results: The mean age of the exposed and control groups are 29.03 ± 3.7 and 29.24 ± 3.5 years respectively. Antioxidant trace metals zinc (64.7 ± 6.09 $\mu\text{g/dl}$), manganese (7.93 ± 1.32 $\mu\text{g/dl}$) and copper (77.8 ± 7.38 $\mu\text{g/dl}$) of the exposed were significantly ($p < 0.001$) lower than the controls (100.2 ± 9.58 $\mu\text{g/dl}$, 9.22 ± 1.19 $\mu\text{g/dl}$ and 103.8 ± 20.09 $\mu\text{g/dl}$ respectively). Selenium (0.03 ± 0.008 $\mu\text{g/dl}$) was significantly ($p < 0.001$) higher in the exposed group compared to the controls (0.01 ± 0.0023 $\mu\text{g/dl}$).

Conclusion: This study has demonstrated lower level of antioxidant trace metals in roadside dispensers of gasoline compared to the controls. This indicates that those exposed are probably at greater risk of developing chronic diseases associated with increase oxidative stress. Enforcing legislation on roadside gasoline dispensing may reduce the risk.

Keywords: Trace metals; occupational exposure; chemical exposure; Gasoline; Gombe.

1. INTRODUCTION

Gasoline as one of the most frequently utilized chemicals. It contributes much of the occupational exposure to toxic chemicals which is a major public health concern worldwide [1], the frequency is increasing due to rapid development in technology [2]. The risk of exposure is even greater in the developing countries, compared to the developed countries. Limited facilities for safe handling of such toxic substances and lack of knowledge of reducing the over exposure and the toxic effects of such chemicals contribute to the increase in the exposure in the developing nations. Poverty has also made people to engage in such occupations without consideration to the health effects of such occupations. Roadside gasoline dispensing is a common occupation in Nigeria most especially in the Northern parts. Lack of gasoline pumps and other protective means may lead to high levels of exposure to gasoline vapors among the roadside gasoline dispensers leading to production of ROS that may consume antioxidants including antioxidant enzymes and their cofactors like antioxidant trace metals.

It is established that gasoline is a very volatile substance, with many organic and inorganic constituents, [1] which when activated, lead to continuous production of reactive oxygen species (ROS) and consumption of antioxidants in the body. This leads to injury and diseases by causing damage to DNA, RNA, and proteins by chemical reactions such as oxidation, nitration, and halogenation leading to genetic modification and alteration in the functions of important lipids, enzymes and other proteins [3-4].

To prevent this injury, the body has developed a robust mechanism using substances known as antioxidants. The antioxidant can either be an enzyme like catalase, superoxide dismutase,

glutathione peroxidase and thioredoxin reductase or a vitamin like beta-carotene, vitamin C and vitamin E. The antioxidant enzymes utilize antioxidant trace metals (selenium, zinc, copper, manganese) as cofactors in reactions leading to detoxification of the ROS in which both the enzymes and their cofactors including antioxidant vitamins and trace metals are consumed [5-7]. The expected increase turnover of the antioxidant enzymes as a response to the toxic ROS generated by exposure to gasoline may lead to the consumption and low levels of the antioxidant trace metals.

In Nigeria, there is paucity of literature on the antioxidant levels among roadside dispensers of gasoline. Therefore, understanding and having knowledge of the levels of antioxidant trace metals among these people will help in generating data to be used in the prevention and timely intervention of chronic gasoline toxicity especially in roadside dispensers of gasoline.

We therefore hypothesized that long-term occupational exposure to gasoline is associated with decreased antioxidant trace metals. The aim of the study, therefore, was to determine and compare the plasma levels of antioxidant trace metals (selenium, zinc, copper, manganese) between roadside dispensers of gasoline and controls in Gombe state, Nigeria.

2. MATERIALS AND METHODS

2.1 Setting

This study is conducted in the department of Chemical Pathology, University College Hospital, Ibadan. The hospital is the premier teaching hospital in Nigeria and serves as a major referral center in Nigeria. The subjects in the study are roadside petrol dispensers in Gombe State, North-East Nigeria. The study was conducted in 2012.

2.2 Design

This is a retrospective cohort study approved by the joint Ethical Review Committee of the University of Ibadan/University College Hospital, Ibadan, recruiting otherwise healthy known roadside dispensers of gasoline in Gombe state using multi-staged sampling method. Age and sex matched controls were consecutively recruited from the same environment.

2.3 Inclusion Criteria

Only apparently healthy, full time roadside dispensers of gasoline that are one year or above in the trade were included in the study.

2.4 Exclusion Criteria

People who have other conditions that can cause oxidative stress were excluded. These include those working in painting, welding, battery, auto mechanics industries [8] and petrol station attendants were also excluded from the study. Patients who have other acute or chronic illness such as diabetes, chronic renal failure and hypertension were excluded from the study. Cigarette smokers and patients taking multivitamins supplements and antiretroviral treatment were also excluded from the study [9-10].

2.5 Selection of Controls

Age and sex matched controls were selected from the general population in the same environment.

2.6 Anthropometric Measurements

Height: - This was measured to the nearest centimetre against a flat, vertical surface with the subjects standing upright. A sliding headpiece was brought to the vertex of the subject's head and the reading at this level was taken.

Weight: - This was taken with salter bathroom scale placed on a flat surface. The reading was recorded to the nearest 0.5 kg. Body mass Index (B.M.I) was then calculated using the formula

$$\text{BMI (kg/m}^2\text{)} = \frac{\text{weight in (kg)}}{\text{Height in (m}^2\text{)}}$$

Their waist circumferences to the nearest 0.5 cm, Hip circumferences, waist to hip ratio and blood

pressure were measured using standard procedures. Random plasma glucose was done using a glucometer. The questionnaires were administered to participants who were asked to fast for sample collection the next morning.

2.7 Sample Collection and Laboratory Procedures

5mls of fasting venous blood was collected from each of the 180 participants into a heparinised plastic tube. Plasma was separated by centrifugation frozen within an hour of collection till the time of analysis.

2.8 Trace Metals Assay

Plasma zinc, selenium, copper and manganese were analyzed using Atomic Absorption Spectrophotometer (AAS) [11].

2.9 Statistical Analysis

The data was analyzed using SPSS version 20.00. Qualitative data were reported using percentages. The mean, standard deviation, skewness and kurtosis were used to measure the normality of distribution of the quantitative variables. The mean (SD) was reported for quantitative data and comparison was made between the cases and controls.

The normally distributed variables were compared between the two groups using student T-test. The level of significance was fixed at the 5% probability level. Pearson correlation coefficient was used to establish correlation between antioxidant vitamins and the duration of exposure among the exposed groups.

3. RESULTS

3.1 Descriptive Statistics

Fifty per cent (90) of my respondents are roadside dispensers of gasoline. Among the controls, 10 (11.1%) are teachers, 9(10%) are farmers, 17(18.9%) are students and 20(22.2%) are other occupations. (Tables 1) Fulanis made up to 103(57.2%) of the respondents while 46(25.6%) are Hausa. Bolewa, Tera and others make the remaining 30(17.2%). Only 2(1.1%) of the respondents take alcohol occasionally and only 15(8.3%) do some exercise. Table 2 shows the duration of exposure among the exposed population. All the continuous variables were

normally distributed and therefore parametric analysis was used.

Table 1. Occupational distribution of the respondents

	Frequency	Percent	Cumulative percent
Gasoline seller	90	50.0	50.0
Trader	34	18.9	68.9
Teacher	10	5.6	74.4
Famer	9	5.0	79.4
Student	17	9.4	88.9
Others	20	11.1	100.0
Total	180	100.0	

Table 2. Average duration of exposure among the exposed population

	Mean	Std. deviation
Duration of exposure (years)	6.4	2.4
Average work hours per day	7.9	0.71
Total exposure (hours)	18332	6931.3

Table 3 shows equality of means in terms of age, blood pressure and other anthropometric characteristics between the cases and controls. This is to remove their confounding effects on the outcome of the study.

3.2 Antioxidants

The mean plasma values of antioxidant trace metals with the exception of selenium, were significantly lower ($P < 0.001$) in the exposed group than the controls. Manganese ($7.93 \pm 1.32 \mu\text{g/dl}$) vs ($9.22 \pm 1.19 \mu\text{g/dl}$), Copper ($77.8 \pm 7.38 \mu\text{g/dl}$) vs ($103.8 \pm 20.09 \mu\text{g/dl}$) and Zinc ($64.7 \pm 6.09 \mu\text{g/dl}$) vs ($100.2 \pm 9.58 \mu\text{g/dl}$). (Table 4)

Selenium was found to be significantly higher ($p < 0.001$) in the exposed group compared to the controls ($0.03 \pm 0.008 \mu\text{g/dl}$) vs ($0.01 \pm 0.0023 \mu\text{g/dl}$) (Table 4)

There was negative correlation between the duration of exposure and antioxidant trace metals with the exception of selenium. Cadmium and lead, similar to selenium showed positive correlation with the duration of exposure. ($p > 0.05$) as shown in Table 5.

4. DISCUSSION

The objective of this study was to evaluate the plasma levels of antioxidant trace metals among roadside dispensers of gasoline. Volatile constituents of gasoline, when inhaled, will lead to continuous production of reactive oxygen species (ROS) and consumption of antioxidants. Since oxidative stress is an imbalance between the rate of production of ROS and their removal by antioxidants, oxidative stress can be assessed by assessing either the increase of production of ROS and its oxidizing effects on proteins, lipids and nucleic acids or by decreased levels of antioxidants [12-13]. This study therefore, compared the plasma levels of antioxidants trace metals between the roadside dispensers of gasoline and controls and also to determine the effect of the exposure period on the antioxidants.

High doses of petrol fumes are expected among roadside dispensers of petrol because they are less protected than petrol station attendants. This is because they use mouth to create a vacuum pressure to dispense the product through pipes into the receivers instead of pumps. They also, almost always, stay by the road side waiting for their customers which in itself is associated with increased levels of exposure to gasoline and its constituents [14].

Table 3. Equality of means of blood pressure and other anthropometric features between exposed and controls

	T test	Mean Dif	P	95% CI	
				Lower	Upper
Age	-0.39	-0.21	0.69	-1.27	0.85
Weight	-1.91	-2.81	0.06	-5.71	0.09
Hight	-0.70	-0.01	0.49	-0.02	0.01
WC	-1.21	-1.47	0.22	-3.86	0.92
HC	-0.90	-0.93	0.37	-2.98	1.12
SBP	-0.47	-9.36	0.64	-48.46	29.73
DBP	-1.21	-1.01	0.23	-2.66	0.64
PR	1.09	9.58	0.28	-7.82	26.98
BMI	-1.76	-0.82	0.08	-1.74	0.10
WHR	-0.89	-0.01	0.37	-0.02	0.01

Table 4. Student's t- test for equality of means between exposed and controls

Variable	Exposed mean (SD)	Controls mean (SD)	P-value
Manganese (ug/dl)	7.93(1.32)	9.22(1.19)	.000
Copper (ug/dl)	77.8(7.38)	103.8(20.09)	.000
Zinc (ug/dl)	64.7(6.09)	100.2(9.58)	.000
Selenium (ug/dl)	0.03(0.0075)	0.01(0.0023)	.000

Table 5. Pearson correlation of duration of exposure (hours) and the trace metals

	r	p
Manganese	-0.171	.107
Copper	-0.120	.260
Zinc	-0.086	.423
Selenium	0.052	.628

Remarkably, with the exception of selenium, this study found a significantly lower level of antioxidant trace metals in the roadside gasoline dispensers than the control groups. This may be because as cofactors of the antioxidant enzymes, manganese, zinc and copper, are continuously utilized to produce the antioxidant enzymes that participate in the detoxification of the ROS [15-16].

Furthermore, previous studies have also reported significantly lower levels of antioxidant enzymes, superoxide dismutase and glutathione peroxidase, and their cofactors including antioxidant vitamins in gasoline exposed subjects than in controls [5,17]. Zinc, manganese and copper, as components of SOD are closely inter-related to antioxidant functions, It is likely, therefore, that their deficiency may lead to impaired free-radical scavenging mechanisms thereby increasing oxidative stress in the exposed group [3,18]. This may explain the increase in the risk of chronic diseases found in this group when compared with controls [19].

The finding in this study, of significantly higher level of selenium among the exposed than the controls is not consistent with what is expected. However, some studies have found higher levels of some antioxidant trace metals in association with low antioxidant enzymes in variable combinations. For example, a study found higher levels of selenium with lower levels of total antioxidant capacity (TAC) among cases (epileptics) compared to controls [20] Similar pattern was observed in a study where selenium was higher despite lower levels in other antioxidants in patients with vitiligo compared with controls in which the significance of higher selenium level was described as unknown [21-

22]. Similar findings were made in many studies [23-26]. Assaying antioxidant enzymes would have given an opportunity to compare the plasma levels of the selenium dependent enzyme glutathione peroxidase (GPx) and the selenium levels in the plasma. This would have predict the possibility of a switch from selenium dependent glutathione peroxidase in the exposed group to the selenium independent isoform which could have accounted for the higher levels of selenium in them [22]. But this is unlikely considering the young age nature of the study participants since the glutathione peroxidase switch is age dependent [22]. Another possibility is by sulphur in the gasoline fumes substituting selenium from selenocysteine which does not happen among the controls [27].

The negative correlation between antioxidant trace metals and the duration of exposure (not statistically significant) further demonstrated the possibility of gasoline mediated decrease in antioxidant trace metals and possibility of increase oxidative stress in the roadside dispensers of gasoline.

There is, therefore, a strong need for going further to find out the effect of antioxidant supplementation on the makers of oxidative stress in the non-gasoline station dispensers of gasoline. This is because many studies have shown a decrease in oxidative stress makers in people with oxidative stress after antioxidants supplementation [28]. If found to be useful; antioxidant supplementation can be advocated to reduce the risk of oxidative stress among the people that are exposed to gasoline.

5. CONCLUSION AND SUMMARY

This study has demonstrated a strong relationship between exposure to gasoline and decrease in antioxidant trace metals. This study may be the first of its kind among roadside gasoline dispensers. There is, therefore, a strong need to do a cohort study that involves following the exposed individuals to monitor progression of the oxidative stress makers or their reduction by antioxidant supplements. From the study, none

of the exposed people use any form of protection, indicating limited knowledge of the risk involved in their occupation. There is, therefore, a great need for public awareness on the risk involved in road side gasoline dispensing and the ways of minimizing it.

CONSENT

The study was explained to the participants in the language they understand and written consent was obtained from them (or other approved parties) for publication of this paper and accompanying images.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Luciano C, Giuseppina IT. Potential health effects of gasoline and its constituents: A review of current literature (1990-1997) on toxicological data. *Environmental Health Perspectives*. 1998;106(3):115-24.
2. Rothman N, Li GL, Dosemeci M, et al. Hematotoxicity among Chinese workers heavily exposed to benzene. *American Journal of Industrial Medicine*. 1996;29:236-46.
3. Georgieva T, Michailova A, Panev T, et al. Possibilities to control the health risk of petrochemical workers. *Int Arch Occup Environ Health*. 2002;75:21-6.
4. Fabiani R, Bartolomeo A, Morozzi G. Involvement of oxygen free radicals in the serum-mediated increase of benzoquinone genotoxicity. *Environ Mol Mutagen*. 2005;46:156-63.
5. Adamu S, Akinosun O, Abbiyesuku F, et al. Evaluation of antioxidant vitamins among roadside gasoline dispensers in Gombe, Nigeria. *J Environ Occup Sci*. 2015;4(3):145-9.
6. Ashour M, Salem S, El-Gadban H, et al. Antioxidant status in children with protein-energy malnutrition (PEM) living in Cairo, Egypt. *Eur J Clin Nutr*. 1999;53(8):669-73.
7. Squali H, Arnaud J, Richard M, et al. Evaluation of oxidative stress and antioxidant defences in malnourished Moroccan children. *Ann Nutr Metab*. 1997;41(3):149-59.
8. Anetor JI, Yaqub SA, Anetor GO, et al. Mixed chemical-induced oxidative stress in occupational exposure in Nigerians. *African Journal of Biotechnology*. 2009;8(5):821-6.
9. Manda KR, Banerjee A, Banks WA, et al. Highly active antiretroviral therapy drug combination induces oxidative stress and mitochondrial dysfunction in immortalized human blood-brain barrier endothelial cells. *Free Radical Biology and Medicine*. 2011;50(7):801-10.
10. Van Antwerpen L, Theron AJ, Myer MS, et al. Cigarette smoke-mediated oxidant stress, phagocytes, vitamin C, vitamin E, and tissue injury. *Annals of the New York Academy of Sciences*. 1993;686:53-65.
11. Arinola G, Idonije B, Akinlade K, et al. Essential trace metals and heavy metals in newly diagnosed schizophrenic patients and those on anti-psychotic medication. *Journal of Research in Medical Sciences*. 2010;15(5):1-5.
12. Myatt L, Cui Z. Oxidative stress in the placenta. *Histochem Cell Biol*. 2004;122:369-82.
13. Sies H. Strategies of antioxidant defence. *Eur J Biochem*. 1993;215:213-9.
14. Navasumrit P, Chanvaivit S, Intarasunanont P, et al. Environmental and occupational exposure to benzene in Thailand. *Chemico-Biological Interactions*. 2005;153-154:75-83.
15. Reena N, Deepti P, Ashok K, et al. Trace elements and antioxidant enzymes associated with oxidative stress in the pre-eclamptic/ eclamptic mothers during fetal circulation. *Clinical nutrition*. 2012;31(6):946-50.
16. Shazia Q, ZH M, Taibur R, et al. Correlation of oxidative stress with serum trace element levels and antioxidant enzyme status in beta thalassemia major patients: A review of the literature. *Anemia*. 2012;2012(2012).
17. Perumalla VR, Mohammed FR, Mohammed M. Genotoxicity in filling station attendants exposed to petroleum hydrocarbons. *The Annals of Occupational Hygiene*. 2010;54(8):944-54.
18. Rossner PJ, Svecova V, Milcova A. Oxidative and nitrosative stress markers in bus drivers. *Mutat Res*. 2007;617:23-32.
19. Adamu S, Akinosun OM, Abbiyesuku FM, et al. Are roadside petrol dispensers at risk of renal dysfunction? A study from Gombe,

- North East Nigeria. Borno Medical Journal. 2015;12(1):16–22.
20. Hamed S, Moustafa M, El-Melegy N. Blood levels of trace elements, electrolytes and oxidative stress /antioxidant systems in epileptic patients. J Pharmacol Sci. 2004;96:465–73.
21. Anne-Marie B, Philippe G, Hélène L, et al. Increase in total blood antioxidant status and selenium levels in black patients with active vitiligo. International Journal of Dermatology. 2002;41(10):640–2.
22. Beazly W, Gaze D, Panske A, et al. Serum selenium levels and blood glutathione peroxidase activities in vitiligo. British Journal of Dermatology. 1999;141:301-3.
23. Yildiz A, Yuksel K, Beran Y, et al. Lipid peroxidation, antioxidant defense, status of trace metals and leptin levels in preeclampsia. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2005;119(1):60 - 6.
24. Bayraktar NM, Karagözler AA, Bayraktar M, et al. Investigation of the blood biochemical status of gas station workers. Toxicological and Environmental Chemistry. 2006;4:587-94.
25. Olaniyi JA, Arinola OG. Essential trace elements and antioxidant status in relation to severity of HIV in Nigerian patients. Med Princ Pract. 2007;16(6):420-525.
26. Zadrožnaa M, Gawlikb M, Nowaka B, et al. Antioxidants activities and concentration of selenium, zinc and copper in preterm and IUGR human placentas. Journal of Trace Elements in Medicine and Biology. 2009;23(2):144-8.
27. Patrick OO, Lewis OA, Nathaniel M, Favour O, John OD, Johanna O, Richard O, Moses I. Total antioxidant status of zinc, manganese, copper and selenium levels in rats exposed to premium motor spirit fumes, North American Journal of Medical Sciences. 2011;3(5):234-237.
28. Zal F, Mostafavi-Pour Z, Amini F, et al. Effect of vitamin E and C supplements on lipid peroxidation and GSH-dependent antioxidant enzyme status in the blood of women consuming oral contraceptives. Contraception. 2012;86(1):62-6.

© 2016 Adamu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

*The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/13507>*